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FUNCTIONAL RECOVERY FOLLOWING ALTERATIONS IN NERVE-MUSCLE CONNECTIONS OF FISHES [1]

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SEVEN FIGURES

Normal motor coordination is not restored in mammals as a rule following regeneration of a severed peripheral nerve trunk. The diffuse and haphazard re-establishment of nerve-muscle connections prevents recovery of normal independent muscle action. Whenever localized contraction of single muscles or muscle groups is called for, there results instead a widespread contraction en masse of the entire re-innervated musculature (Sperry, '45).

Quite different is the picture of recovery in larval amphibians and in the one species of fish (*Sphaeroides spengleri*) so far investigated. Excellent recovery of motor coordination has been found in the former after regeneration of the limb and very early oculomotor nerves (Weiss, '36; Sperry, '47), and in the latter after regeneration of the pectoral fin nerves (Sperry, '50). How the normally timed, individuated muscle function is recovered after peripheral disarrangement of nerve-muscle connections remains to be determined. As a possible explanation it has been suggested that the synaptic terminals on the regenerated motor cells may undergo some kind of chemotrophic break-down in the centers followed by regeneration of new central hook-ups patterned to suit the altered peripheral innervation (Sperry, '41, '51a, '51b). A

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synaptic interchange of this sort would be in harmony with various other phenomena that point to specific chemoaffinities as a basis for the orderly formation and maintenance of synaptic patterns in the vertebrate nervous system. However, morphological or other direct evidence for such synaptic readjustment is lacking.

For further analysis of the problem the fishes seem to offer the most promising possibilities. Mammals are eliminated and presumably also birds and reptiles by the low level or absence in postdevelopmental stages of this type of functional recovery. Among the amphibians and fishes the technical difficulties of staining the synaptic terminals appear to be somewhat less formidable in the fishes. Further, motor recovery was found to be more rapid and complete in the one species of adult fish studied than that obtained in adult amphibians.

The present experiments were undertaken with reference to the following questions: (a) Is the motor plasticity reported for the pectoral system of *S. spengleri* (Bloch) present also in other teleosts, specifically the closely related *S. testudineus* (Linnaeus) and the distantly related and highly specialized *Histrio histrio* (Linnaeus)? (b) What happens when the pectoral fin musculature is reinnervated by nerves of the pelvic fin or by pectoral nerves with greatly reduced fiber count? (c) Does a similar plasticity prevail in the cranial oculomotor nuclei? This latter question is particularly important with reference to the search for morphological evidence of the postulated synaptic readjustment, because the conditions for histological demonstrations of possible synaptic changes are much more favorable in the cranial nuclei than in the cord. Included also are the results of some preliminary microscopic observations of the motor cells of brain and cord of *S. testudineus* following peripheral nerve section and regeneration.

MATERIALS AND METHODS

Specimens of *H. histrio,* the sargassum fish, ranging in total length from 3.4 to 4.7 cm were collected in the Gulf Stream off

Pectoral system

Direct regeneration, S. testudineus. The large nerve of the pectoral fin musculature was completely divided on one side in 4 adult specimens 18 to 22 cm in length. The nerve was exposed via an abdominal approach and severed by repeated pinching and teasing with jeweler's forceps. The incision was closed with two silk sutures that were removed approximately 4 days later. Complete motor paralysis of the affected fin was evident in all 4 cases during the first two weeks after nerve section.

Beginning on the 17th–19th days after operation the fins began to exhibit slight twitching movements. Motor recovery advanced rapidly during the subsequent several weeks until the operated and unoperated sides were indistinguishable. From the beginning the fin ray movements seemed to be properly timed. There was no period of incoordination or mass action, or other indication that the restoration of function involved learning. The period of complete paralysis lasted only a few days longer than in the small *S. spengleri* and the final recovery at 5 weeks was fully as good.

Direct regeneration, H. histrio. This species is extremely specialized particularly with respect to its pectoral fin, the morphology and movement patterns of which are analogous to and approach in complexity those of the tetrapod limb. If the capacity for restoring motor coordination as observed in *S. spengleri* and its close relative, *S. testudineus* has been lost through evolution anywhere among the teleosts, it seemed likely that it might be absent in the pectoral system of *H. histrio.*

Some sketches of the musculature and skeleton of the fin of *H. histrio* are provided in figure 1. Although incomplete in detail, the sketches indicate the complexity of the muscle anatomy and function involved. The limb as a whole can be swung in any direction from its base, and also can be rotated on its long axis through approximately 120°. The individual fin rays can be swung back and forth, spread apart, or pulled

fin rays tend to wrap about an object such as a glass rod when it comes into contact with the ventral surface of the fin, and (d) miscellaneous paddling, pulling, and supporting movements. In all cases the posture and coordination of the operated fin were compared closely with those of the unoperated fin on the opposite side.

In a group of 10 fish the nerves supplying the pectoral fin on the left side were completely transected through a lateral incision over the cleithrum. The cut nerve ends were frayed with forceps in a rough manner so as to prevent any systematic restoration of the original innervation pattern. In 5 of these cases the nearby pelvic nerves were sectioned along with the pectoral in the same operation. The pelvic fins of *H. histrio* also have a rather specialized musculature, but not so elaborate as that of the pectorals.

Paralytic immobility and insensitivity of the affected fins during the week immediately following the operation indicated complete nerve severance in all cases. One case died on the 4th day. Signs of motor and sensory recovery began to appear in the remaining fish about 10 days after operation and became progressively more pronounced during the following several weeks. At 5 weeks after operation good motor coordination, approaching closely that on the normal side, was evident in both the pectoral and pelvic fins.

Recovery was good in this series but not perfect. Minor asymmetries between left and right fins were still evident in 7 of the 9 cases at the termination of the experiments, 53 days after operation. For example, the fin rays in some instances did not spread quite so widely nor so evenly as on the normal side, or the "elbow" in other instances was bent a little more than normal. These slight defects seemed more readily ascribed to incompleteness of recovery than to any positive dysfunction of misregenerated fibers.

One of the cases with both the pectoral and the pelvic nerves sectioned was exceptional in that the pectoral recovery was retarded and showed an exaggeration of protraction in the resting position characteristic of crossed pelvic reinnervation

as described below, though less pronounced. At postmortem dissection it was found in this case that two strands of fibers had crossed from the pelvic nerve scar into the nearby pectoral nerves.

Reduction of proximal nerve supply. In a second group of 7 *H. histrio,* the pectoral nerve trunks were completely sectioned. The anterior fascicles constituting from one-half to two-thirds of the total nerve supply were then reflected dorsally and the cut ends were embedded in the dorsal trunk musculature as a means of preventing their regeneration into the fin muscles. The cut ends of the remaining nerve fascicles were apposed to the cut ends of the combined fascicles of the total distal nerve trunk. The source of reinnervation was thus greatly reduced in total quantity and probably changed considerably in quality with many of the fibers to the more anterior fin muscles having been eliminated.

Two of the 7 cases in this series died during the initial period of complete paralysis. Functional recovery in the remaining 5 proceeded much as in the first group but was less rapid and complete. However, by the end of the 6th week all 5 fish had regained good general use of their fins. The withdrawal reflex, planing, grasping, and various paddling movements were recovered in good order. Asymmetries remained more pronounced than in the first group, but their nature in these cases also was such as to suggest weakness of muscle function rather than complete afunction or positive dysfunction.

The fish were sacrificed at the end of the 6th week and later were dissected with the aid of a stereoscopic microscope. In one case it appeared that the reflected central ends of the pectoral nerves had pulled loose and some fibers had regenerated back into the pectoral fin. In a second case, the presence of diffuse fibers between the reflected central nerve stumps and the fin left some question as to whether elimination of the reflected fibers had been complete. In the remaining three cases the nerve supply to the fin remained markedly reduced in the proximal region as intended.

In both groups of *Histrio,* the operated fin had lagged behind the opposite normal fin in growth. This was especially marked during the second week after nerve section. After its reinnervation the operated fin regained gradually its normal proportion until at the end of 6 weeks little or no size difference remained. The *Histrio* were growing fairly rapidly during these 6 weeks and had increased in over-all length approximately two to two and one-half times.

Cross-union of pelvic to pectoral nerves. In a third group of 10 *H. histrio* the central ends of the divided nerves of the pelvic fin were cross-united to the distal ends of the divided nerves of the pectoral fin. The central ends of the pectoral nerves were then reflected dorsally and inserted into dorsal trunk musculature to prevent their regeneration. The intention was to effect a re-innervation of the pectoral fin musculature by the nerves of the pelvic fin.

On the 16th day after operation signs of beginning functional recovery were evident in the 6 cases still alive. (Four cases were killed by an accident in the water system during the second week after operation.) By the 20th day the pectoral fin had become extremely flexed at the "elbow" in all 6 cases and the fin as a whole was abnormally protracted until the distal end of the fin rays touched or nearly touched the head below the eye. The individual fin rays remained closely drawn together. The pelvic fins meanwhile had assumed a slightly elevated posture and active movement was absent.

Mechanical stimulation of the pectoral fin on the 20th day elicited a weak response of this same fin amounting only to an exaggeration of the above pose that pulled the fin farther forward against the head and pursed more tightly the fin rays. The normal response is much the reverse involving extreme retraction of the fin caudally against the tail, with supination and spreading of the fin rays. Postmortem dissection of two cases on the 22nd and 24th days after operation respectively revealed that the nerves had been nicely crossed as intended in both animals with the bulk of the pelvic fibers having regenerated into the pectoral fin. The superficial flexor and

protractor muscles appeared to have undergone a permanent contractural shortening correlated with the persistent protractor posture of the fin.

By the 26th day the pelvic fins in the remaining 4 cases had come to assume a more normal posture, and in three cases the pelvic fin had begun to recover some active movement which appeared to be normal in direction and timing. Distinct crossed responses of the pelvic fin upon stimulation of the pectoral fin were noted along with the pectoral reactions in all 4 cases on the 32nd day. In one animal, pelvic responses occurred upon stimulation also of the pelvic fin. In another, stimulation of the pelvic fin evoked crossed reactions in the pectoral fin in the form of clear-cut adductor twitches of the upper fin rays. The pectoral fin response to pectoral stimulation seemed weaker than in earlier stages of recovery.

Following these behavioral observations the effects of electrical stimulation of the nerves and of the pectoral muscles were studied in the latter 4 cases. An inductorium was used and the fish were lightly anesthetized with urethane. The pelvic and pectoral fin nerves were first sectioned far proximally. Stimulation of the pelvic nerves usually evoked some response in both fins, the strength varying in different instances. The pectoral responses were very restricted and consisted only of fin protraction and weak twitching of the fin rays. The pelvic response was also weak and consisted largely of depression of the base of the pelvic fin. No reactions were obtained from proximal stimulation of the reflected pectoral nerves.

Direct stimulation of the pectoral muscles yielded protraction primarily with some elevation and depression, but no retraction. All the pectoral fin muscles appeared thin and atrophic with the possible exception of the protractors which had undergone contracture. The pectoral fin as a whole was stunted to approximately two-thirds normal size. The pelvic fin by contrast was more nearly normal in size, posture, and movement insofar as the latter was present. Although a few pelvic fibers were found to have crossed into their original distal

pathways in all 4 cases, the bulk of the pelvic fibers remained connected to the pectoral fin as intended.

Electrical stimulation of the bared pectoral fin nerves in two normal anesthetized *H. histrio*, led predominantly to protraction of the pectoral fin accompanied by closure of the fin rays, i.e., this massive electrical stimulation caused the fin to move into much the same posture as that which prevailed chronically after its re-innervation by the pelvic nerves.

Histological observations. In an additional series of 16 adult *S. testudineus* the pectoral fin nerve was sectioned on one side. These fish were then sacrificed and fixed individually for histological study at spaced intervals from two to 27 days after nerve section. The pectoral segments of the cord were sectioned serially. Both the Cajal and Rasdolsky stains were used as described above. No marked differences were noted between operated and unoperated sides during the first week after nerve section. Beginning about the 8th day after surgery the motor cells in the pectoral columns on the operated side appeared swollen (figs. 2, 6). The swelling was more pronounced during the third week after operation and was still evident on the 27th day, the longest interval examined. The cell nucleus was somewhat enlarged along with the cell body proper, and also the proximal regions of the dendrites (fig. 6). It was not possible to tell whether there was any degeneration or regeneration of synaptic endings in either the Cajal or Rasdolsky preparations. This is not unexpected in view of the extreme density and fine calibre of the terminal fibers in the spinal neuropil of the adult fish (fig. 7).

Oculomotor system

In amphibians motor recovery following regeneration of the oculomotor nerve, unlike that following regeneration of the motor nerves of the limbs, is generally poor both in urodeles and in anurans even in larval stages (Sperry, '47). Only in extremely early larval or post-embryonic stages (19 mm *Rana grylio* larvae) was good recovery obtained comparable

to that found in the limb system in much later larval and even adult stages. This difference between oculomotor and pectoral systems proved to be equally marked in *S. testudineus*.

In 10 adult *S. testudineus* the oculomotor nerve was sectioned intracranially through a large dorsolateral opening in the skull. Most of the intracranial portion of the trochlear nerve was extirpated in the same operation leaving only the lateral rectus muscle to move the eyeball. Two of these cases died from unknown causes during the second postoperative week and 4 more were killed the following week by an accident in the water system. Dissection of the latter 4 revealed that a large bridge of regenerated fibers had already been established between the proximal and distal nerve stumps.

In three of the remaining 4 cases the normally-innervated lateral rectus muscle was excised on the 28th day after operation because the movements effected by this single muscle were extensive and would have obscured any minor activity that might have been recovered in the reinnervated muscles. After removal of the normally innervated abducent muscle, all eye movements were reduced to weak twitches the excursions of which were so minute that it was not possible to determine whether their direction and timing were normal. Except for a slight medial tilt, the posture of the eye during movement and at rest was normal. The lateral rectus muscle was removed in the 4th case on the 41st day after nerve section with the same result as in the first three cases. All 4 animals were fixed 46 days after section of nerve III. At this time there still had been no discernible improvement in eye movement.

Anatomical observations. After removal of the perfused brain, the head was immersed in 10% formalin and the extraocular muscles and their nerves were later dissected. The denervated trochlear muscle was reduced to approximately one-half its normal size. The muscles reinnervated by the oculomotor nerve, on the other hand, were not reduced more than one-fourth at the most and for the majority of the mus-

cles no definite size difference was discernible between operated and unoperated sides.

The oculomotor muscles were fixed in 10% formalin and stained by the formic acid-gold chloride method. The reinnervated muscles stained much more intensely than the normal and appeared to contain more connective tissue. However, microscopic measurements of the muscle fiber diameters failed to reveal any significant shrinkage. It was not possible to make out details of the nerve terminals nor myoneural junctions.

The brains of two of the experimental cases were treated with the Rasdolsky stain and the other two with the Cajal silver method. Five additional cases were studied in which the eyeball was extirpated on one side including all its extrinsic eye muscles at 8, 12, 13, 17, and 23 day intervals before fixation. The 13 day case was treated with the modified Rasdolsky stain and the others by the Cajal method.

The pronounced retrograde enlargement observed in the pectoral motor cells of the cord was not found in the oculomotor and trochlear nuclei (see figs. 3, 4, 5). If any minor swelling of the motor cells was present, it was obscured by the normal variation in cell size and by the bilateral distribution of the affected neurons in nucleus III correlated with the partial decussation of the oculomotor root fibers (Edgeworth, '35). However, in the 4 experimental cases there was a definite difference between normal and affected sides in the appearance of the nerve roots. In the Cajal preparations the fibers tended to be fewer in number, thinner, and less nearly parallel on the affected side, and the outlines of the myelin sheaths were less regular. In the Rasdolsky preparations the root fibers on the affected side stained less intensely and the root bundles were densely pervaded with glial and other cells containing large vacuoles. These degenerative changes seemed no more pronounced and were possibly less marked in the trochlear root fibers which presumably failed to re-establish functional connections of any sort in the periphery.

DISCUSSION

The good-to-excellent recovery of motor coordination obtained after section and regeneration of the pectoral fin nerve in both *H. histrio* and *S. testudineus* is consistent with the previous findings in *S. spengleri* (Sperry, '50). The results furnish further evidence that this type of motor plasticity is more highly developed in adult teleosts than in adult amphibians. Absolute size, next to phylogenetic status and developmental age, has also been considered a possible factor influencing neural plasticity and functional recovery after regeneration. In this connection it is noteworthy that recovery in the largest *S. testudineus* was as complete and almost as rapid as in the much smaller *S. spengleri*.

That good restoration of function was also obtained after extensive reduction in the proximal supply of pectoral nerve fibers in *H. histrio* gives further indication that the recovery involves a spread of specific influences from the muscles to their motoneurons which in turn determines the incidence of central discharge as inferred to explain the comparable recoveries in amphibian limb coordination (Wiersma, '31; Weiss, '36).

The reasons for the lack of good recovery in the pelvic-to-pectoral crosses are not evident from the data and may have been associated primarily with any one or a combination of the following: (a) failure of the regenerating pelvic fibers to form adequate transmissive connections with the pectoral muscles, (b) incapacity of the pelvic fibers to undergo the respecification required to suit the pectoral musculature, (c) lack of adequate synaptic readjustments in the spinal centers. From the reflex responses elicitable after regeneration it is clear that cutaneous fibers of the pelvic fin re-established functional connections in the skin of the pectoral fin and also that a small fraction, at least, of the pelvic motor fibers were able to form transmissive endings in pectoral muscle. Some of these sensory and motor fibers, possibly all, retained their original reflex relations in the centers. There were indications that the pelvic motor fibers did not re-establish functional

connections as readily with pectoral as with pelvic musculature in that the recovered pectoral movement remained extremely weak and the muscles showed signs of atrophy. The pelvic musculature, by contrast, was restored in comparatively good condition even though it was re-innervated by only a comparatively few stray pelvic fibers.

In the case of oculomotor regeneration the functional result was not strictly comparable to anything we have observed previously in fishes, amphibians or mammals. The results approached somewhat the functional picture that would be expected if only those axons that happened to connect with their original muscles were able to form functional neuromotor endings. However, the recovered function was even weaker than would be expected on this basis wherein approximately one-fourth the original number of motoneurons should have been functioning normally. If one assumes that the establishment of atypical nerve-muscle relations tended to produce an adverse effect on the entire motoneuron involved, even though other terminal branches of the same axon were properly connected, and that each regenerating fiber underwent several bifurcations in the scar region, then one might expect approximately the type of result observed. As in the pelvic-to-pectoral crosses, the results suggest a degree of functional incompatibility between motor fibers and strange muscles to which they normally do not connect. To what extent this incompatibility is expressed through failure to form adequate transmissive connections in the periphery and to what extent the failure is in the centers, it is not possible to determine from the present data. The observed muscular atrophy associated with the lack of functional recovery may itself have been an effect of either peripheral or central dysfunction.

Our failure in these exploratory observations to find direct microscopic evidence for a switching of central fiber connections in correlation with functional restoration in the pectoral musculature is not to be taken as an indication of its absence. Synaptic relations in the neuropil of the teleost cord are

largely beyond demonstration with currently available histological technique. In the cat where synaptic endings are somewhat easier to see, degeneration of synaptic terminals around chromatolyzed motoneurons following section of motor axons has been reported by Barnard ('38, '40), but the finding has not been consistently confirmed (Barr, '40; Schadewald, '41). The occurrence of degeneration and also regeneration of synaptic endings around chromatolyzed cells in the cat cord has since been inferred from the changes in reflex potentials that follow section of motor axons (Campbell, '44; Downman, et al., '53).

The present findings point up new complexities in the relations of the vertebrate motoneuron to its muscle fibers on the one hand and its synaptic end-feet on the other. However, the problem of how the central timing of misregenerated motor fibers becomes readapted in fin and limb segments of the cord to suit abnormal nerve-muscle connections remains unanswered.

SUMMARY

Recovery of normal muscle coordination was observed following surgical section and regeneration of the nerves supplying the pectoral fin in *S. testudineus* and in *H. histrio*. Microscopic examination of the pectoral region of the cord revealed a marked enlargement of cell bodies and dendrites of the sectioned motor fibers, but nothing was detected to indicate breakdown and re-establishment of synaptic terminals. Good recovery was observed also in *H. histrio* after the anterior one-half to two-thirds of the sectioned pectoral fibers were prevented from regenerating by reflecting them into nearby trunk musculature. However, only abnormal contractures resulted when pelvic fin nerves were crossed surgically to re-innervate the pectoral fin.

Section of the oculomotor nerve in *S. testudineus* was followed by good regeneration, but there was no recovery of muscle action. In microscopic preparations of the oculomotor

nuclei the oculomotor neurons did not exhibit the retrograde enlargement observed in the cord and the root fibers appeared atrophic.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

Photomicrographs of 8 μ sections of cord and midbrain of *S. testudineus*. All except figure 2 are from Rasdolsky preparations.

2 Through upper segments of cord showing pronounced enlargement of motor cells in pectoral column on operated (right) side at 23 days after nerve section. Cajal. $\times$ 30.

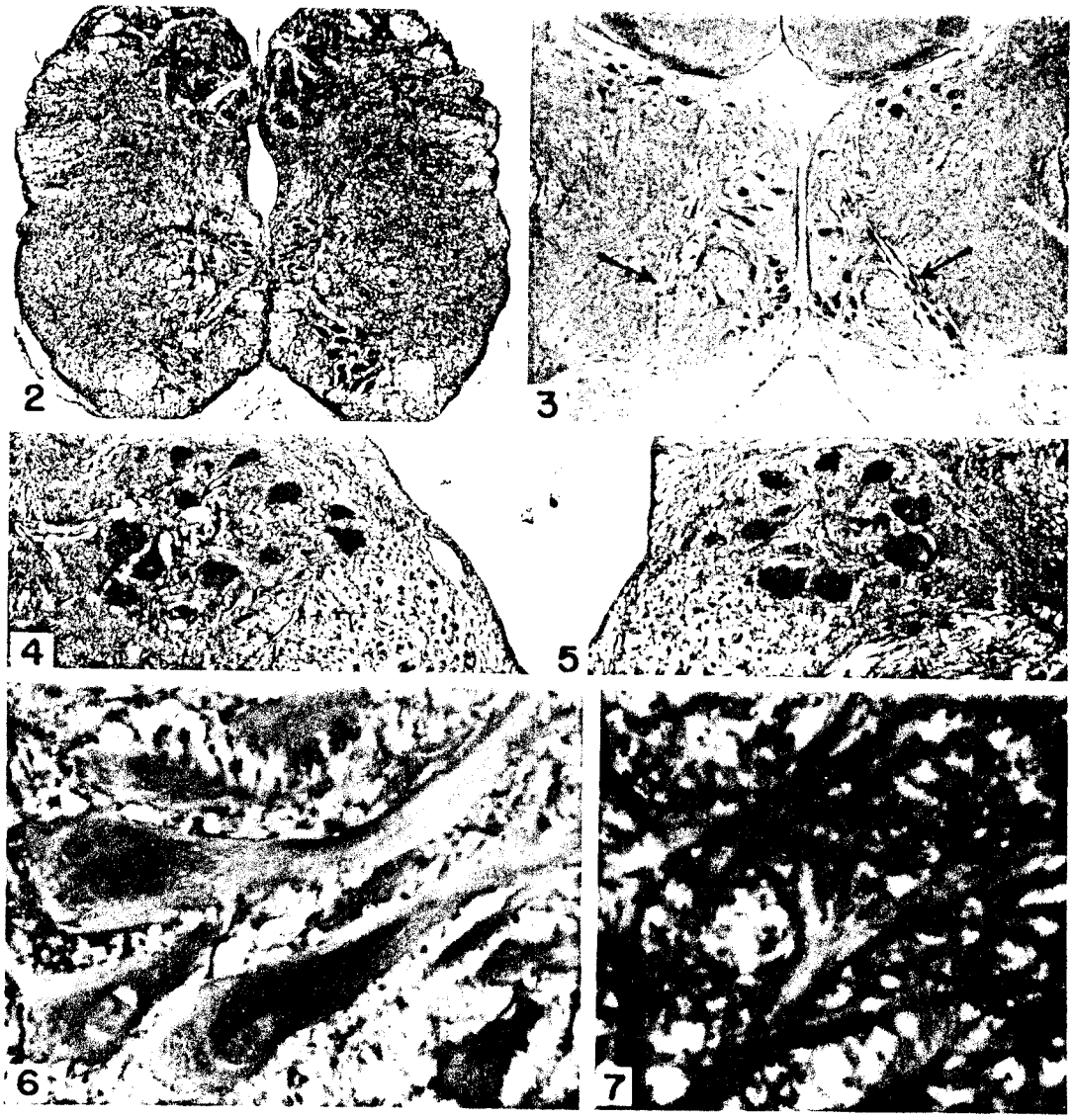
3 Through oculomotor nuclei showing lack of comparable swelling in oculomotor cells and atrophic appearance of affected (left) root fibers (arrows) 44 days after nerve section. $\times$ 45.

4 Trochlear nucleus 44 days after orbital resection of trochlear nerve. $\times$ 115.

5 Normal trochlear nucleus from same section as figure 4. $\times$ 115.

6 Enlargement of pectoral motor cells at 16 days after nerve section. $\times$ 350.

7 View of dendritic field of cells shown in figure 6. Other than a size increase in thicker portions of dendrites on affected side, no differences were detected between dendritic fields of normal and sectioned motor nerves. $\times$ 450.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